

AMENDMENTS TO THE SPECIFICATION:

Please replace paragraph beginning at page 1, line 12 with the following amended paragraph:

The present invention generally relates to a plant nitrogen regulatory PII gene (hereinafter P-PII gene), a gene involved in regulating plant nitrogen metabolism. The invention provides P-PII nucleotide sequences, expression constructs comprising said nucleotide sequences, and host cells and plants having said constructs and, optionally expressing the P-PII gene from said constructs. The invention also provides substantially pure P-PII proteins.

Please replace paragraph beginning at page 4, line 20 with the following amended paragraph:

The present invention relates to a plant nitrogen regulatory P-PII gene involved in regulating nitrogen assimilation in plants. The invention provides P-PII coding nucleotide sequences, expression constructs comprising P-PII coding sequences, and host organisms, including plants, containing said expression constructs. The invention also provide P-PII proteins.

Please replace paragraph beginning at page 8, line 13 with the following amended paragraph:

Figure 1. Comparison of the deduced amino acid sequences of plant PII and microbial PII polypeptides. Figure 1A. The amino acid residues conserved between all microbial PIIs and plant PII are shaded. The asterisks (*) indicate residues conserved between plant and any bacterial PII. Boxed areas labeled I and II include residues of two PII signature domains conserved between all PIIs. The position of the tyrosine residue (Tyr 51) which is uridylylated in *E. coli* is indicated by an arrow. ~~Genus-species abbreviations for glnB genes encoding PII and references:~~ The PII sequences shown in this figure are: At, *Arabidopsis thaliana* (SEQ ID NO:1); Ric, *Ricinus communis* (SEQ ID NO:2); Kp, *Klebsiella pneumoniae* (SEQ ID NO:3); Ec, *Escherichia coli* (SEQ ID NO:4); R1, *Rhizobium leguminosarum* (SEQ ID NO:5); Bj, *Bradyrhizobium japonicum* (SEQ ID NO:6); Az, *Azospirillum brasilense* (SEQ ID NO:7); Rc, *Rhodobacter capsulatus* (SEQ ID NO:8); Sy, *Synechococcus* (SEQ ID NO:9) strain PCC 7942; Mtl, *Methanococcus thermolithotrophicus*

glnB-like protein 1 (SEQ ID NO:10) Mt2, *Methanococcus thermolithotrophicus* glnB-like protein 2 (SEQ ID NO:11). For best alignment of Mt2 with glnB, the last two residues, BN, and the peptide sequence FSANLPEIVDIQKII (SEQ ID NO:12) are deleted. This deletion is indicated by "^" in the Mt2 sequence. The numbers 1, 51, and 112 indicate the residue positions of the E. coli PII protein. Figure 1B. The relationship between plant PII and microbial PIIs is shown by a phylogenetic analysis using parsimony. Bacillus PII-like protein is an anomaly which does not cluster with bacterial taxa. F, phenylalanine; Y, tyrosine.

Please replace paragraphs beginning at page 11, line 15 with the following amended paragraphs:

Figure 12. Nucleotide sequence of *Arabidopsis* P-II cDNA clone (SEQ ID NO:13).

Figure 13. Nucleotide sequence of *Ricinus* Castor Bean P-II cDNA clone (SEQ ID NO:14).

Please replace paragraph beginning at page 13, line 23 with the following amended paragraph:

The nucleotide coding sequences ~~and deduced amino acid sequences~~ for *Arabidopsis* and castor bean P-II are depicted in FIGS. 12 and 13, respectively. These nucleotide sequences, or fragments or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of the P-II gene product, or functionally active peptides or functional equivalents thereof, in appropriate host cells.

Please replace paragraph beginning at page 14, line 10 with the following amended paragraph:

The P-II nucleotide sequences of the invention include (a) the DNA sequence in FIG. 12 (SEQ ID NO:13); (b) the DNA sequence spanning from nucleotide 33 to 620 as shown in FIG. 12 (SEQ ID NO:15); (c) the DNA sequence in FIG. 13 (SEQ ID NO:14), (d) the DNA sequence spanning from nucleotide 50 to 643 as shown in FIG. 13 (SEQ ID NO:16); (e) any nucleotide sequence that hybridizes to the complement of the DNA sequence shown in FIG. 12 and encodes a functionally equivalent product; and (f) any nucleotide sequence that hybridizes to the complement of the DNA sequence shown in FIG. 13 and encodes a functionally equivalent product. Functional equivalents of the P-II include

naturally occurring P-II in other plant species, the mutant P-II whether naturally occurring or engineered. The invention also includes degenerate variants of sequences (a) through (f).

Please replace paragraph beginning at page 15, line 4 with the following amended paragraph:

The P-II nucleotide sequences of the present invention also include any nucleotide sequence encoding a plant protein containing the amino acid sequences designated At or RIC in ~~FIG. 1~~ FIG. 1A; and (b) any nucleotide sequence that hybridizes to the complement of the DNA sequences that encode the amino acid sequence designated At or RIC in FIG. 1. The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore complements of the nucleotide sequences (a) and (b). Such hybridization conditions may be highly stringent or less highly stringent. In addition to the P-II nucleotide sequences described above, full length P-II cDNA or gene sequences present in the same species and/or homologs of the P-II gene present in other plant species can be identified and readily isolated, without ~~undue~~ undue experimentation, by molecular biological techniques well known in the art. The identification of homologs of P-II in related species can be useful for developing plant model systems for purposes of discovering activators or inhibitors of P-II to modify P-II in plants to alter plant nitrogen metabolism. Alternatively, such cDNA libraries, or genomic DNA libraries derived from the organism of interest can be screened by hybridization using nucleotides described herein as hybridization or amplification probes.

Please replace paragraph beginning at page 17, line 18 with the following amended paragraph:

The P-II gene sequences may additionally be used to isolate mutant P-II gene alleles. Such mutant alleles may be isolated from plant species either known or proposed to have a genotype which contributes to the plant growth ~~rate~~ rate or nitrogen metabolism. Mutant alleles and mutant allele products may then be utilized in the therapeutic and diagnostic systems described below. Additionally, such P-II gene sequences can be used to detect P-II gene regulatory (e.g., promoter or promotor/enhancer) defects which can affect plant growth.

Please replace paragraph beginning at page 29, line 29 with the following amended paragraph:

In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the P-P_{II} coding sequence ~~is~~ is inserted within a marker gene sequence of the vector, recombinants containing the P-P_{II} coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in tandem with the P-P_{II} sequence under the control of the same or different promoter used to control the expression of the P-P_{II} coding sequence. Expression of the marker in response to induction or selection indicates expression of the P-P_{II} coding sequence. Two marker gene constructs which may be of particular-value for monitoring promoter activity in plant cells and plants are the bacterial glucuronidase gene, GUS (Jefferson et al., 1987, EMBO J. 6:3901-3908) or the luciferase gene (Ow et al., 1987, Science, 234:856-859).

Please replace paragraph beginning at page 37, line 22 with the following amended paragraph:

A λ ZAPII library containing cDNAs from developing endosperm and embryos of castor (*Ricinus communis* L.) seeds was used for mass-sequencing (van de Loo et al., 1995, Plant Physiol.). Out of 743 clones sequenced, one clone showed sequence similarity to nitrogen-regulatory protein P_{II} of *Synechococcus* and other bacteria (Tsinoremas et al., 1991, Proc. Natl. Acad. Sci. USA 88:4565-4569). This 0.85 kb cDNA called pRc-GLB1 was used to make a ³²P-dCTP labeled probe by random priming and used to screen an *Arabidopsis* genomic library at a reduced stringency as follows. The hybridization was performed in QuickHyb (Stratagene) at 50°C for 1 hr. Filters were washed with 2xSSC, 0.1% SDS, 5 min twice at room temperature followed by 0.5x SSC, 0.1% SDS wash at 50°C for 30 min. One *Arabidopsis* genomic GLB1 clone with a 3 kb DNA insert was isolated. A 1.1 kb NcoI-NcoI DNA fragment of the genomic clone which strongly hybridized to the castor GLB1 cDNA was used to screen an *Arabidopsis* silique cDNA library. Under high-stringency hybridization conditions, two *Arabidopsis* cDNA clones were obtained from 2x10⁵ plaques. These *Arabidopsis* GLB1 cDNAs were sequenced using the Sequenase method (U.S. Biochemical Corp.). DNA sequences were analyzed by the GCG Sequence Analysis

software package (Genetics Computer Group Inc., Madison, WI). 5' RACE was performed using Gibco-BRL 5' RACE system. Reverse transcription was performed with 1 μ g total RNA from light-grown plants. Nested primers for RACE were MH7 (GCAAGATGGTCGGGAATGTC) ([SEQ ID NO:17](#)), MH8 (CGACAGGTAAAACACGACTG) ([SEQ ID NO:18](#)) and MH9 (GGTCTGACAATTGCTTCCAC) ([SEQ ID NO:19](#)). PCR conditions were 94 °C 2 min, followed by 30 cycles (94°C 20 sec, 55°C 30 sec, and 72°C 40 sec). PCR products were subcloned using the pCR-Script kit of Stratagene.

Please replace paragraph beginning at page 38, line 21 with the following amended paragraph:

Arabidopsis genomic DNA was isolated according to the procedure described by Ausubel et. al, 1987, In: Current Protocols in Molecular Biology, (Greene Publishing Assoc. & John Wiley & Son), except that the buffer used was 8.75 M urea, 438 mM NaCl, 62.5 mM Tris-HCl pH 8.0, 62.5 mM EDTA. RNA was isolated using a phenol extraction protocol (Jackson, A.O. and Larkins, B.A., 1976, Plant Physiol. 57:5-10). Southern blot and Northern blot were performed as described (Sambrook et al., Molecular cloning: a laboratory manual, 3rd ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory)). For detection of *GLB1* mRNA, membranes were hybridized with single-stranded DIG-labeled probes made from *pAt-GLB1* cDNA using PCR (Myerson, D., 1991, Biotechniques 10:35-38). To generate probes approximately 540 nucleotides in length covering the PII coding region and partial 3' non-coding region, MH5 (GAAACCAAACACAGACTCC) ([SEQ ID NO:20](#)) and MH6 (CCGAGTAATAACAGTCGTC) ([SEQ ID NO:21](#)) primers were used. The control DIG-labeled probes for *GLN2*, *P-PIIN1* and 18 rRNA were made by random priming (Boehringer Mannheim).